

DOCKET NO.: TIBO-0029
Application No.: 09/836,477
Office Action Dated: November 2, 2005

PATENT



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Brendan Larder, Stuart Bloor, Kurt Hertogs,
Pascale Alfons Rosa Dehertogh and Rudy
Jean Marc Mortier

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Application No.: 09/836,477

Group Art Unit: 1631

Filing Date: April 18, 2001

Examiner: Lori A. Clow

For: Methods for Measuring Therapy Resistance

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION PURSUANT TO 37 C.F.R. 1.131

We, Kurt Hertogs and Rudy Jean Marc Mortier, declare as follows:

1. We are two of the five named inventors in the above-identified application.
2. We have read the specification and the claims as originally filed in the application and as presented in the pending claims shown in the attached Exhibit A. Each of us, together with Brendan Larder, Stuart Bloor and Pascale Alfons Rosa Dehertogh, contributed to the conception of the invention as defined by one or more of the claims as set forth in Exhibit A.
3. Before March 2000, we completed the invention in this country, or in a NAFTA country, or a WTO member country. Our actual reduction to practice of the claimed invention, directly or through persons under our direction and control, before March 2000, is evidenced by the following:
 - A. Attached as Exhibit B is a true and accurate copy (except for redaction of dates) of witnessed report, prepared by Kurt Hertogs ("the Hertogs Report"). Hertogs memorializes the claimed invention as shown in Exhibit A. The Hertogs Report at Exhibit B, is dated prior to March 2000.

B. The Hertogs report discloses in Exhibit B at page 4, ¶1.1, the "System and System Features to be tested" which discloses the steps as claimed in pending claim 1.

C. The Hertogs report discloses in Exhibit B "VircoGen II, i.e., the prediction of genotypic resistance based on available phenotypic data." (see Exhibit B at page 4, ¶1.1).

D. The Hertogs report in Exhibit B at page 4, ¶1.1, describes, in detail, new steps to validate the calls based on phenotypic data. The new steps include:

1. Create Hot Spots from rules, or use a set of predefined Hot Spots (preferred) (see Exhibit B at page 4, ¶1.1).
2. Import a reference set of genotypic and phenotypic data (AV_Data). The program will identify sequences belonging to each Hot Spot and link them to the Hot Spots (see Exhibit B at page 4, ¶1.1).
3. From the Hot Spots "Special" button, recalculate the Phenotypic Sets. This will link the set of corresponding phenotypes to each Hot Spot (see Exhibit B at page 4, ¶1.1).
4. For each test sequence, a report is created using the new method to determine genotypic resistance. A set of "Profiles" is automatically calculated for each drug. A profile consists of a set of Hot Spots (either positive or negative). To belong to a profile, a test sequence must have an identical profile. The mean and median phenotypic resistance are also calculated for each Profile (see Exhibit B at page 4, ¶1.1).

E. The Hertogs report discloses in Exhibit B at page 8 examples of drugs used in the method as claimed.

F. The Hertogs report discloses in Exhibit B at page 8 the following note: "he[sic] phenotypes and sequence data should be imported, the hot spots should be correct and the phenotype set should be calculated before starting the test script."

G. The Hertogs report discloses in Exhibit B at page 21 various fields in an Excel file which include: sequence identifier; drug (compound tested), fold resistance observed in the antivirogram linked to a sequence; phenotypic call for the real data; and original virtual fold resistance.

H. The Hertogs report discloses in Exhibit B at page 24 under the header "7. Test Summary Log" the following: "Verify the scoring of genotypic calls in the VircoGenTM database (virtual phenotypes)".

I. The Hertogs report in Exhibit B therefore shows in detail all the steps to be performed to arrive at the result as claimed in Exhibit A.


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4. All statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true. These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

January 26th 2006
Date

27 January 2006
Date


Kurt Hertogs


Rudy Jean Marc Mortier

489338

EXHIBIT A

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FILED APRIL 18, 2001**

PENDING CLAIMS

I. Listing of Claims:

1. **(Previously Presented)** A method of determining a phenotype of a retrovirus, wherein the retrovirus is the Human Immunodeficiency Virus, comprising:
 - a) obtaining a genetic sequence of the Human Immunodeficiency Virus;
 - b) identifying a mutation pattern of the genetic sequence of the Human Immunodeficiency Virus, wherein said mutation pattern comprises at least one mutation that correlates to resistance to at least one therapy;
 - c) searching a relational genotype/phenotype database for at least one database mutation pattern similar to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus;
 - d) obtaining at least one database phenotype of the at least one database mutation pattern; and
 - e) determining the phenotype of the Human Immunodeficiency Virus from the at least one database phenotype.
2. **(Original)** The method of claim 1, wherein a series of phenotypes is obtained by repeating steps b) through e) for each therapy in a group of therapies.
3. **(Previously Presented)** The method of claim 1, wherein said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus is specific to a therapy.
4. **(Previously Presented)** The method of claim 1, wherein the Human Immunodeficiency Virus is obtained from at least one of a plasma sample, a blood sample, a saliva sample, mucous sample, and a tissue sample.

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5-7. **(Canceled)**

8. **(Previously Presented)** The method of claim 1, wherein said at least one mutation is chosen from a frame shift mutation, a base substitution, and an epigenetic mutation.

9-12. **(Canceled)**

13. **(Previously Presented)** The method of claim 1, wherein the genetic sequence of Human Immunodeficiency Virus is the genetic sequence of the protease region of the Human Immunodeficiency Virus genome, the genetic sequence of the reverse transcriptase region of the Human Immunodeficiency Virus genome, or the genetic sequence of the protease region and reverse transcriptase region of the Human Immunodeficiency Virus genome.

14-15. **(Canceled)**

16. **(Previously Presented)** The method of claim 1, wherein said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus comprises at least two mutations that correlate to resistance to at least one therapy.

17. **(Original)** The method of claim 1, wherein the search of the relational genotype/phenotype database for at least one sample with a similar mutation pattern uses cluster searches.

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PENDING CLAIMS

18. **(Previously Presented)** The method of claim 1, wherein the database mutation pattern comprises at least one mutation found in said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus.

19. **(Previously Presented)** The method of claim 1, wherein the database mutation pattern is a mutation pattern in which at least about 50% of the mutations are identical to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus.

20. **(Previously Presented)** The method of claim 19, wherein the database mutation pattern is a mutation pattern in which at least about 80% of the mutations are identical to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus.

21. **(Previously Presented)** The method of claim 20, wherein the database mutation pattern is a mutation pattern in which at least about 90% of the mutations are identical to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus.

22. **(Previously Presented)** The method of claim 21, wherein the mutations of the database mutation pattern are identical to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus.

23. **(Previously Presented)** The method of claim 1, wherein the phenotype of the Human Immunodeficiency Virus is a mean fold-change in resistance, wherein said mean fold change is obtained from all of the database phenotypes obtained in step d).

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24. **(Previously Presented)** The method of claim 1, wherein the phenotype of the Human Immunodeficiency Virus is expressed as an IC₅₀.

25. **(Previously Presented)** A method of reporting a phenotype for a Human Immunodeficiency Virus, comprising generating a report having the phenotype determined using the method of claim 1.

26-27. **(Canceled)**

28. **(Previously Presented)** A method of determining a phenotype of a retrovirus, wherein the retrovirus is the Human Immunodeficiency Virus comprising:

- a) obtaining a genetic sequence of the Human Immunodeficiency Virus;
- b) searching a relational genotype/phenotype database for at least one database genetic sequence similar to said genetic sequence of the Human Immunodeficiency Virus;
- c) obtaining a database phenotype of the at least one database genetic sequence; and
- d) determining the phenotype of the Human Immunodeficiency Virus from the database phenotype.

29. **(Previously Presented)** The method of claim 28, wherein the at least one database genetic sequence is at least about 60% identical to the genetic sequence of the Human Immunodeficiency Virus.

30. **(Previously Presented)** The method of claim 29, wherein the at least one database genetic sequence is at least about 70% identical to the genetic sequence of the Human Immunodeficiency Virus.

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31. **(Previously Presented)** The method of claim 30, wherein the at least one database genetic sequence is at least about 80% identical to the genetic sequence of the Human Immunodeficiency Virus.

32. **(Previously Presented)** The method of claim 31, wherein the at least one database genetic sequence is at least about 90% identical to the genetic sequence of the Human Immunodeficiency Virus.

33-38. **(Canceled)**

39. **(Previously Presented)** A computer program for determining a phenotype of a retrovirus, wherein the retrovirus is the Human Immunodeficiency Virus, wherein the program is comprised on a computer readable medium, comprising:

- a) receiving a genetic sequence from the Human Immunodeficiency Virus from a patient;
- b) identifying a mutation pattern of the genetic sequence of the Human Immunodeficiency Virus, wherein said mutation pattern comprises at least one mutation that correlates to resistance to at least one therapy;
- c) searching a relational genotype/phenotype database for at least one database mutation pattern similar to said mutation pattern of the genetic sequence of the Human Immunodeficiency Virus;
- d) obtaining at least one database phenotype of the at least one database mutation pattern from the relational genotype/phenotype database;
- e) determining the at least one phenotype of Human Immunodeficiency Virus from

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the at least one database phenotype; and

f) providing the phenotype of the Human Immunodeficiency Virus sample.

40. **(Original)** The computer program of claim 39, wherein a series of phenotypes is obtained by repeating steps b) through e) for a group of therapies.

41. **(Previously Presented)** The computer program of claim 40, wherein the phenotype of the Human Immunodeficiency Virus is provided in a report.

42. **(Canceled)**



VIRCO

**Test Script for the Validation of
VIRIS.**

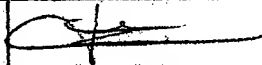



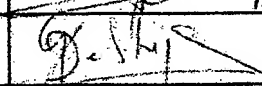
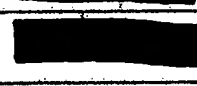
**Laboratory Management
Information System (LIMS)**

TS-VG-Genotype Calls

Edition Nr 3

IT IS FORBIDDEN TO COPY THIS DOCUMENT

KODÉE

	Name	Signature	Date
Author	Frank Peeters		
Reviewer	Kurt Hertogs		
Approver	Guido De Schrijver		

Revision History		
Version	Date	Reason
1	[REDACTED]	Validation of the Genotypic calls in VircoGen
2	[REDACTED]	Validation of VircoGen II
3	[REDACTED]	Validation of data cleaning routine

11/24/2014

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1. Purpose

1.1 System and System Features to be tested

The present Test Script verifies the core functionality of VircoGen II, i.e. the prediction of genotypic resistance based on available phenotypic data. The analysis is based performed on the same data set used to validate VircoGen™ (rule-based interpretation) The latter validation consisted of three steps :

- Alignment of reference and test sequences
- Selection of the "documented" mutations (i.e. the mutations known or suspected to cause resistance)
- Rule-based analysis of the documented mutations. The genotypic data were scored as follows :
 - No evidence of resistance (green score)
 - Resistance possible (orange score)
 - Evidence of resistance (red score)

The new steps to validate the calls based on phenotypic data include :

- Create Hot Spots from rules, or use a set of predefined Hot Spots (preferred)
- Import a reference set of genotypic and phenotypic data (AV_Data) . The program will identify the sequences belonging to each Hot Spot link them to the Hot Spots.
- From the Hot Spots "Special" button, recalculate the Phenotype Sets. This will link the set of corresponding phenotypes to each Hot Spot.
- For each test sequence, create a report using the new method to determine genotypic resistance. A set of "Profiles" is automatically calculated for each drug. A profile consists of a set of Hot Spots (either positive or negative). To belong to a profile, a test sequence must obey to all of the positive Hot Spots, and may not belong to any of the negative Hot Spots. The Mean and Median phenotypic resistance are also calculated for each Profile.

In addition the routine to clean the data based on the 3sigma levels needs to be validated.

All calculations must be performed in VircoGen II, and in parallel using a dedicated Excel file that performs identical calculations, or a statistical package such as Statistica.

1.2 Reference Documentation

The documents references in this Test Script are the related Validation Plan and Test Plan, which are based on the EU Annex 11 Guidelines.

Other reference documentation includes the VIRIS User Manual and the System Design Specifications which are used to optimise the computer program during the testing and implementation phase.

2. Special Requirements

Special Requirement	Description
Prerequisite procedures	<ul style="list-style-type: none"> • Preparation of a genotype-phenotype analysis programs in Excel : • - Profile Template.XLS • - Pheno-Spread Template.XLS • Report_Data Template.XLS
Special skills required	<ul style="list-style-type: none"> • Thorough knowledge of VircoGen II, Excel and Statistica • Knowledge of the 4D Quick Report Editor
Environment requirements	<ul style="list-style-type: none"> • Power PC with at least 64 Mb of internal memory • IBM compatible computer running Statistica

3. Test Approach

This section describes the approach to be followed for :

1. Specifying the tests (Test Script).
2. Executing the Test Script and completing the Test Log.
3. Resolution of unexpected events.

3.1 Test Preparation

A Test Script consists of a number of steps that are executed sequentially. Each step specifies the action to be performed, the results that are expected, and the pass/fail criteria for the test. The steps to be executed are specified in section 4 of this Test Script.

3.2 Test Execution Procedure

1. The Test Script is also the template for the Test Log. Before executing the Test Script, a copy of this Test Script is made and the title is changed to identify it as appropriate Test Log.
2. When a step is executed, the actual result is entered in the Test Log to mark its completion.
3. For each step, the observed and expected results are compared. The tester writes "pass" or "fail" in the space provided, depending on the outcome.
4. Section 5 of the Test Log describes all errors and all anomalies observed. Anomalies are any observations of system behaviour that is not expected.
5. Section 6 of the Test Log describes notes that are required to interpret correctly the observed behaviour of the system. Abnormal environmental conditions are also noted. Test criteria that are not set correctly may require that updated criteria are used and the test continued. This will also be explained by means of an explanatory note.
6. At the completion of all procedure steps, a Test Summary is written.

3.3 Resolution of Unexpected Events

Anomalies are noted and discussed with the IT Director who will perform further actions. These actions have to be documented in an "Anomaly and Change Request Form".

4. Test Script Steps & Test Log

Instructions for Building the Test Script

- Describe any preparation that is required before the test can be carried out
- Describe the set of input data that are used (test case).
- Describe the Test Procedures. A Test Procedure may consist of a number of steps that are performed in a specified sequence
- Describe the steps necessary to accomplish the Test Procedure. Write exactly what the tester will do and what will be the input to this step
- List the results that are expected or refer to an appended sheet or printout. There may be a number of expected results, depending on the complexity of the test you are performing.
- State what are the pass/fail criteria for the tests.
- The length of the test procedure will depend on the complexity of the test to be performed.

The following table describes the various steps to be performed to test the feature or set of features that will be tested in this test script. The Test Log entries and the list of anomalies (=unexpected results) are completed when executing the script.

SEQUENCES AND PROFILES Test Procedures		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Start VircoGen II	Main screen and menu appear			
Open File – Test Sequences	The main view of the sequences table appears.			
For a test sequence from Test Case 1, execute the "Analyse Sequences" program from the "Special" button. Select the "Resistance" analysis. Copy the result screen to the clipboard.	The screen can be pasted in Word. File created : "Rule Analysis Results"			
Open the "Interpretation Settings" and set the "Minimum amount of matches needed for interpretation" to a value of 1000 (above the number of sequences in the database).	When creating a report, the Database Pheno Spread will not be calculated yet.			
Open the Test Sequences and make a report for the sequence you are working on. To do this, use the "Special" button "Create Reports". Respond "No" to the question "Save Analysis Data".	An R in front of the Sequence indicates that the report has been generated. The profiles are NOT saved to the table "MedianFR_Values".			
Go to the Reports table and print the report on paper.	Report is printed. All results should be calculated using the rules (Rule-based interpretation).			
Compare the genotype calls obtained from the "Analysis" (in Rule Analysis Results Excel file) with the results on the reports.	The results should be identical, since they are both obtained using a rule-based interpretation.			
Open the Excel file: "Profile Template.XLS" and copy the sequence you are working on in row 2 of the "sequences" worksheet.	The 2 nd worksheet (Virco ID) is updated with the new sequence information			
Select the rows for which the In Report value (Column J) is TRUE.	A subset of rows containing documented mutations is shown.			
Compare the mutations with the mutations on the picture of the report printed from VircoGen II Add the Mixtures and inserts in the box under the Drug and Mutations columns (if needed).	The mutations should automatically be highlighted in yellow in the Hot Spots below.			
Manually score the Hot Spots. Put 1 or 0 depending if the sequence obeys the Hot Spot or not.	The "Excel Profile" in the "Profile" worksheet should be filled out correctly.			
Print the "Virco ID" worksheet.	The worksheet is printed in landscape on two pages (one for the sequence data and one for the Hot Spots).			
In VircoGen II, open the Test Sequences and make a new report for the sequence you are working on. To do this, use the "Special" button "Create Reports". Respond "Yes" to the question "Save Analysis Data".	New reports are generated for the sequences. The profiles are saved to the table "MedianFR_Values".			
Open the table "MedianFR_Values" and	The profiles are saved to a text file.			

export the drug name and profile (using the Quick Report Editor behind the Print button).	Archive these files in a folder.			
Go to the "Profiles" tab of the Excel file and paste the drug and profile in the first two columns of the sheet.	The drug s should be pasted in the following order : Saquinavir Ritonavir Indinavir DMP-266 Delavirdine Nevirapine Nelfinavir 1592 U89 3TC d4T ddC AZT ddI			
Compare the profile obtained by 4D (pasted) with the profile calculated using Excel. If everything is OK, a green value "TRUE" will be displayed in the last column of the "Profiles" sheet. If not, a red "FALSE" will appear.	The profiles from Excel should be identical to the scores from the VircoGen™ program. (after correction of eventual manual errors).			
Repeat this analysis for each Test Sequence from Test Case 1 or quit VircoGen™, Excel and Word.	New analysis executed or quit programs.			

Test Case 1 :

The following 28 sequences (Virco lds) were used as test cases :

101968	102636	103076
102360	102648	103084
102364	102657	103110
102382	102660	103111
102448	102663	103129
102459	102691	103173
102488	102718	105521
102611	102736	110126
102631	103067	
102635	103070	

Note : he phenotypes and sequence data should be imported, the hot spots should be correct and the phenotype set should be calculated before starting the test script.

INTERPRETATION SETTINGS Test Procedures		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Start VircoGen II	Main screen and menu appear			
Open File – Test Sequences	The main view of the sequences table appears.			
Open the "Interpretation Settings" and set the "Minimum amount of matches needed for interpretation" to a value of 1000 (above the number of sequences in the database).	When creating a report, the Database Pheno Spread will not be calculated yet.			
Open the Test Sequences and make a report for the sequence your are working on. To do this, use the "Special" button "Create Reports". Respond "No" to the question "Save Analysis Data".	An R in front of the Sequence indicates that the report has been generated. The profiles are NOT saved to the table "MedianFR_Values".			
Go to the Reports table and make a print of the Detail Screen (using F12 with Flash-it active)..	Detail screen is printed. All results should be calculated using the rules (Rule-based interpretation).			
Open the "Interpretation Settings" and set the "Minimum amount of matches needed for interpretation" to a value of 1.	When creating a report, the Database Pheno Spread will be used when at least one result is available.			
Open the Test Sequences and make a report for the sequence your are working on. To do this, use the "Special" button "Create Reports". Respond "No" to the question "Save Analysis Data".	A new report is generated. The profiles are NOT saved to the table "MedianFR_Values".			
Go to the Reports table and make a print of the Detail Screen (using F12 with Flash-it active)..	Detail screen is printed. All results should be calculated using the profiles (when phenotype data are available).			
Compare if the common data are identical.	Identical data : - Mutations picture - Drug name - "Mutations identified" flag - Matching rule - Resistance call (SIR) - Mean FR - # Sequences in set - # Phenotypes in set - Remarks			
Repeat this analysis for each Test Sequence from Test Case 1 or quit VircoGen™, Excel and Word.	New analysis executed or quit programs.			
Create the same data using the research tool "Engine Test". (This is not a required test). Open the Test Sequences table, select all records and execute "Test VG II Engine" under the Special button. Print the test data (Consolidation – Performed Tests) and detailed records (Consolidation – Test Results).	Verify the results. They should match the data in the Reports of the sequences (screen dumps described above). A calculation is also performed to show the differences between the Real Medians obtained in the Antivirogram™ test, and the results from the Pheno spread. Ideally, no "level two" differences should be observed.			

Test Case 1:

Same data as above.

PHENO SPREAD Test Procedures		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Start VircoGen II.	Main screen and menu appear			
Open File – Hot Spots	The main view of the hot spots list appears.			
Select all hot spots corresponding to one drug via the 'Search' button (see below for ID numbers).	The hot spots list contains all hot spots for the drug you selected. ID's should be in ascending order.			
Select a hot spot and open File – Test sequences.	The test sequences window appears displaying all test sequences corresponding to the hot spot you selected.			
Select all test sequences and export their VircoID's using the Quick Report behind the 'Print' button.	Tab-delimited text file printed (temporary file).			
Paste the VircoID's in the 'profiles' tab of the "Pheno-spread template.xls" Excel file. Use the correct column of the Profile window (column 1 to 9).	Virco IDs pasted in correct column of Profile window.			
Do the same steps for every hot spot for the drug you're working on.	All Virco IDs copied into Profile window.			
From the 'Mean and Median 28 sequences' tab in the Pheno-spread Excel file select the drug you are working on. Print the result. Note : this tab was imported from VircoGenII.	Printed copy with name of the drug, profiles and mean and median FR.			
In the 'Profiles' tab fill in the first profile in the yellow profile row. Print this page.	The Result column identifies all VircoID's of the test sequences that are positive for that profile (identified by Yes).			
Select the 'AV_data' tab and filter the drug column for the correct drug and the "In profile" column for Yes	The FR and Resistance level are displayed in the Patient_FR column and the Res_level column respectively.			
Paste the data from the latter two columns to the A and B columns under the header 'Table'. Print the resulting page. This is needed because otherwise "hidden" results will be used to calculate the Means, Median and Resistance values.	Mean and median are calculated, counts and percentages for low, medium and high level resistance are given are presented numerically and graphically. The profile results are printed for that drug.			
Do the same for each profile for the drug you are working on.	All Profiles calculated and printed.			
For all profiles, compare the mean and median n FR calculated in Excel with the mean and median FR in the 'Mean and Median 28 sequences' tab.	All results have to be the same.			
For the profile 000000000 look up all corresponding Virco ID's and their phenotypic data (patient FR). The latter you find in the AV_data list that you access via the File_Show phenotypic data menu in VircoGenII. Calculate the mean and median FR and compare with the mean and median FR in the 'Mean and Median 28 sequences' tab. Write the results on the printed copy with name of the drug, profiles and mean and median FR.	The mean and medians obtained using the manual method should equal the data obtained by 4D.			

Exhibit B

VIRIS Computer System Validation

VIRIS

Repeat this analysis for each drug or quit VircoGenII, Excel and Word.	All results OK or quit VircoGen II.			
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Test Case 1 :

Same data as above.

Note : drug IDs :

- 1 : Saquinavir
- 2 : Ritonavir
- 3 : Indinavir
- 4 : DMP266
- 5 : Delavirdine
- 6 : Nevirapine
- 7 : PMEA (do not test)
- 8 : Nelfinavir
- 9 : 1592 U89
- 10 : 3TC
- 11 : D4T
- 12 : DDC
- 13 : AZT
- 14 : DDI
- 2756 : VX-478 (do not test)

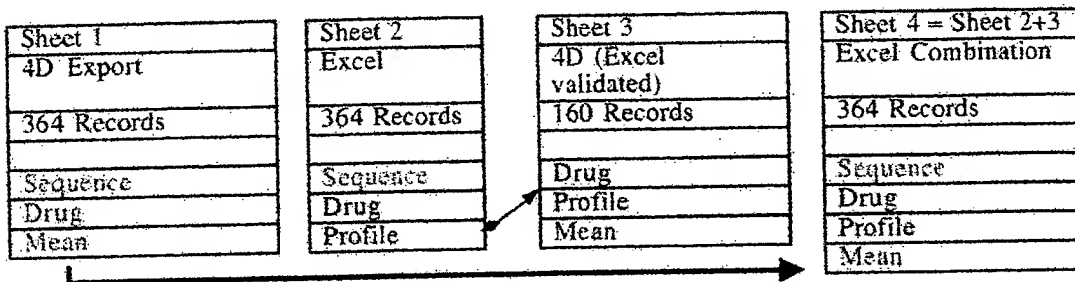
4D - EXCEL COMPARISON Test Procedures		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Start VircoGen II	Main screen and menu appear			
Go to "Report_data" in the user environment. Export at least the following fields : - Virco ID - Generic Name - Resistance (rule-based call) - Mean_FR	A Tab-delimited text file is generated.			
Import this file in the Excel file "Report_Data 28 Template.xls" and rename the file "Report_Data 28 sequences.xls". Make a column "Drug" that holds the preferred name.	Sheet 1 contains the Mean_FR (obtained from the pheno spread analysis) for each combination of Virco ID - Drug (364 records).			
Open the Excel file "nnnnn.xls" in the folder "Step 1 - Profiles" and extract the following information from the "Profiles" worksheet: - Virco ID - Drug - Profile Paste this information in Sheet 2 of the file "Report_Data 28 sequences.xls".	Sheet 2 tells to which profile each combination of "Virco ID - Drug" belongs. This means that the mutations observed for that sequence (Virco ID) belong to these sets of Drug - Profiles (364 records)			
Go to the "MedianFR_Values" table in VircoGen II and export all the data to a tab-delimited text file : - Drug - Profile - Mean_FR - Median_FR Import this file in Sheet 3 of the Excel file "Report_Data 28 sequences.xls". Make a column that concatenates the Drug and Profile fields.	Sheet 3 shows the Mean and Median FR values for each Drug - Profile (160 records).			
In Sheet 4, combine the information from sheets 2 and 3 to create a 364-record table containing the following information : - Virco ID - Drug - Profile - Mean FR - Resistance (based on Pheno spread) Make a column that compares the Mean FR values between Sheet 4 and Sheet 1.	This table should now contain the same information as in Sheet 1. The difference is that this sheet was generated using Excel data, while sheet 1 was exported from 4D.			
Make a column that compares the resistance calls between Sheet 4 and Sheet 1.	Verify the difference. These indicate a difference between the rule-based interpretation (VG I) and the interpretation based on the pheno-spread (VG II). It is not the intention of this validation to discuss the differences.			

Test Case 1 :

Same data as above.

Note :

Graphical presentation of the steps performed in this script to verify the means:



STATISTICS Test Procedures (1)		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Start VircoGen II using the production database containing the correct Hot Spots, and all available genotypic and phenotypic data of the Virco database.	Main screen and menu appear. The database should contain about 20000 genotypes and 350000 AV_Data.	pass		
Clear all Analysis results and MedianFR Values tables.	Tables should be empty.	pass		
Go to the Hot Spots and recalculate all phenotype sets using the menu "Recalculate phenotype sets" from the "Special" button.	The sets should be recalculated	pass		
Import the sequences from Test Case 1.	The 28 sequences should be imported, and they should automatically be linked to the correct Hot Spots.	pass		
Create reports for these sequences. Save the analysis results. Print the reports.	The 28 reports should be generated and printed.	pass		
Go to the Research menu "Consolidation - Show Saved Analysis". Select a record from Test Case 2 (Virco ID - Drug) and open the AV_Data table using the menu "File - Phenotype Data". Export all records using the Quick Report Editor (under the Print button). Export the following data : - Virco ID - Drug - Patient FR - Resistance level	All records exported into files.	pass		
Repeat the step above for all drugs of the Virco IDs of Test Case 2.	All records exported into files.	pass		
Combine all the exports for one Virco ID into one Excel file. Add a column containing the Log10(FR)	Two files should be generated (one for each of the test cases described in Test Case 2).	pass		
Import the file into the statistical program "Statistica" and perform the following statistical analysis per drug : - Descriptive statistics - Frequency tables - Plot Histograms For the Fold resistance, the Resistance level and the Log of the fold resistance	The statistics are performed and printed. Compare the results with the data obtained from 4D : - Mean fold resistance - Number of records for each of the Resistance classes	pass		
Repeat the previous two steps with the other Excel file.	The statistics are performed and printed. The results obtained using 4D and Statistica are identical.	pass		
Quit Statistica, Excel and 4D.	All programs closed.			

Test Case 1 :

Same 28 sequences as used above.

These are imported in a database containing the complete set of genotypes and phenotypes from the Virco database.

Test Case 2 :

All drugs of Virco ID : 102611
 103076

STATISTICS Test Procedures (Correctness of data cleaning)		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Open Statistica and use Test Case 1	Statistica opened and data available	pass		
Perform descriptive Statistics to obtain means and standard deviations (on the log values)	Statistics printed	pass		
Calculate the 3-sigma limits (mean \pm 3 * standard deviation) of the log values	3-sigma limits calculated and introduced in Excel file	pass		
Make a copy of the data set	Data set copied	pass		
Delete the cases outside the 3-sigma limits (for each drug)	Outliers manually removed	pass		
Perform the statistics : - descriptive statistics - frequency tables - histograms	Statistics performed and printed	pass		
Compare the means obtained using the latter analysis with the original means	All data should be identical	pass	1	
Perform the same validation for Test Case 2.		pass	1 3	

Test Case 1 :

All drugs of Virco ID : 103076
 Name copy : 103076s3

Test Case 1 :

All drugs of Virco ID : 102611
 Name copy : 102611s3

STATISTICS Test Procedures (Effect of data cleaning)		Test Log		
Procedure / Steps	Expected Result	Pass Fail	Note Log	Anomaly Log
Use two separate 4D servers and start Test Cases 1 and 2.	Two 4D servers started.	Pass		
Go to the Hot Spots and recalculate all phenotype sets using the menu "Recalculate phenotype sets" from the "Special" button.	The sets should be recalculated on both servers.	Pass		
Open the Sequences table, select data from Test Case 3 and start the routine "Test Engine" (from the Special Menu).	The Test Engine selects the sequences for which both genotypic and phenotypic data are present and estimates the virtual phenotypes for these samples (on both servers).	Pass		
Go to the Research menu "Consolidation - Median_FR values". Export all records using the Quick Report Editor (under the Print button). Export the fields described in test case 3.	Fields exported to Tab-delimited text files (about 10000 records on both servers).	Pass		
Create reports for the first 30 sequences. Save the analysis results. Print the reports.	The reports should be generated and printed (on both computers : original set and cleaned set).	Pass		
Quit VircoGen II and open the two exported files in Excel. Combine the results in a pairwise manner so that the real, original and cleaned results for each drug are displayed on one line. Insert a column that calculates the difference between the "original" Call and the real Call, and between the "cleaned" Call and the real Call.	An Excel file containing the columns described in "Test Case 4". The 2 additional columns are calculated.	Pass Pass		
Import the file into the statistical program "Statistica" and perform the following statistical analysis : - Descriptive statistics on all data - Correlations on FR data - Multiple regression on FR data - Descriptive statistics on call differences	The statistics are performed and printed. A correlation exists between the real data and the virtual calls. The cleaned results show a higher correlation than the original data.	Pass		
Create new Statistica files in which the data are transformed, so that a BY analysis can be performed. This means that the dependent variables are not in separate columns, but in one column with an accompanying Classification column. Classify the data by CLASS (Real, Original or Cleaned). Generate on file per drug.	Statistica files generated per drug. The classification variable CLASS can be used to perform statistics BY class, and to generate categorised plots.	Pass		
Perform the following statistical analysis on each of the datafiles (drug files) : - Descriptive statistics - Frequency tables - Plot Histograms - Box & Whisker plots on the Fold resistance data, Calls and Call differences (Matches).	All resulting listings and graphs are printed.	Pass	2	

Exhibit B

VIRIS Computer System Validation

VIRIS

Quit Statistica, Excel and the 4D servers.	All programs closed.	Jan		
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Test Case 1 :

Program : VircoGen II without "Cleaning" module
 Data : Complete copy of Virco UK production database.
 Latest version of rules and hot spots.
 Empty Reports, Median FR table and Phenotype results table.

Test Case 2 :

Program : VircoGen II containing the "Cleaning" module based on the 3-sigma levels
 Data : Complete copy of Virco UK production database.
 Latest version of rules and hot spots.
 Empty Reports, Median FR table and Phenotype results table.

Test Case 3 :

Sequences for which an Antivirogram exists.
 Make random selection of about 1000 Sequences.
 (note : the internal memory allows that maximum 1200 sequences can be computed at once).

Fields to be exported :

Virco ID	Sequence identifier
Drug	Compound tested
FR	Fold resistance observed in the Antivirogram linked to a sequence
CALL	Phenotypic call (S,I,R) for the real data

Test Case 4 :

Fields in Excel file :

Virco ID	Sequence identifier
Drug	Compound tested
REAL_FR	Fold resistance observed in the Antivirogram linked to a sequence
REAL_CALL	Phenotypic call (S,I,R) for the real data
ORI_FR	Original VIRTUAL fold resistance (before data cleaning)
ORI_CALL	Original VIRTUAL phenotypic call (before data cleaning)
ORI_MATCH	Difference between ORI_CALL and REAL_CALL
CLEAN_FR	VIRTUAL fold resistance obtained after cleaning of the data
CLEAN_CALL	VIRTUAL Call obtained after cleaning of the data
CLEAN_MATCH	Difference between CLEAN_CALL and REAL_CALL

5. Test Execution Notes

Test Notes					
Notes Log NR	Description				
1	<p>Apparently some means were different between statistics and 40:</p> <table border="1"> <tr> <td>103076</td><td>sequencing</td></tr> <tr> <td>102611</td><td>relative response</td></tr> </table> <p>For use due to manual errors during the clearing.</p> <p>Manual recalculation gave the <u>correct</u> results.</p>	103076	sequencing	102611	relative response
103076	sequencing				
102611	relative response				
2	<p>Results in statistical report</p> <p>ST - Input II Data clearing</p>				
3	<p>Statistical file too large (not 16000 entries)</p> <p>⇒ 2 files generated 53 35</p>				

6. Anomalies observed

Anomaly Log	
Anomaly Log NR	Description

7. Test Summary Log

Summary of Tests			
Test Procedure	Date Performed	Initials	Pass/Fail
- Verify the scoring of genotypic calls in the VircoGen™ database (virtual phenotypes)	[REDACTED]	PP	Pass

8. Tester & Witness Signature and Date

Name	Title	Signature	Date
Tester: F. Peeters	SEA Administrator	[Signature]	[REDACTED]
Witness: K. Muly	Lab. Director	[Signature]	[REDACTED]

9. Annexes

Number of pages : Statistical report
 ST - VircoGen II data cleaning
 + Statistics data & graphs

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